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Intervention Study Comparing Blood NAD+ Concentrations with Liposomal and Non-Liposomal Nicotinamide Mononucleotide

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1. Abstract

It is now known that lifestyle-related diseases are caused by senescence. The International Classification of Diseases, 11th Edition (ICD-11) has coded the item "related to aging," and aging has come to be considered a disease. Therefore, mitochondrial activity is currently attracting attention as a way to prevent aging. Our research team screened various substances and found that nicotinamide mononucleotide (NMN) is effective in mitochondrial activity. However, there are many NMN products on the market, and issues remain regarding their content and unconfirmed effectiveness. Therefore, in this exploratory clinical study, liposomal NMN, non-liposomal NMN for 4 weeks, 350 mg/day of NMN, and a placebo were administered in a double-blind manner, and the levels of nicotinamide adenine dinucleotide (NAD+) were measured. As a result, the expression level of NAD+ was significantly higher in liposomal NMN, and we will report on its effects and underlying mechanism.

2. Introduction

It is known that many lifestyle-related diseases such as cancer, dementia, myocardial infarction, and stroke are caused by aging [1]. In 2022, the WHO's disease classification system, ICD-11, added the code "aging-related" [2]. While chronological aging is irreversible, senescence is reversible. It has become a target for medical intervention and treatment aimed at promoting healthy longevity and preventing other diseases. Mitochondrial activity is attracting attention in this regard [3]. It is believed that senescence can be prevented by increasing mitochondrial activity [4]. However, there is currently no established method for measuring mitochondrial activity itself. Mitochondria are a type of organelle that produces adenosine triphosphate (ATP) as an energy source. ATP is a substance necessary for activity in the body to produce energy by hydrolyzing itself, so if it can be produced efficiently, it may be useful for preventing lifestyle-related diseases and senescence [5]. Measurement of NAD+ is currently attracting attention as a biomarker of increased mitochondrial activity itself [6]. It is believed that measuring NAD+ level can quantitatively measure the degree of aging, which is also correlated with disease risk [7].NAD+ is an essential substance for energy production, necessary to maintain vital activities. It is also known to promote the expression of genes that prevent aging, such as the sirtuin gene, often referred to as the 'longevity gene' [8]. Furthermore, it has been reported that NAD+ is related to many characteristics of aging [9]. Blood NAD+ concentration decreases with age, which leads to a decline in organ and tissue function, as well as an increased risk of aging-related diseases [11,12]. For this reason, blood NAD+ concentration is thought to be useful as a biomarker for diagnosing

the degree of aging and mitochondrial function.NAD+ is an electron carrier used in eukaryotes [12]. It has a function such as a coenzyme for various dehydrogenases and exists in two forms: oxidized (NAD+) and reduced (NADH) [13]. NAD+ is a substance consisting of nicotinamide mononucleotide and adenosine, with the 5' end of each nucleotide bound by a phosphate bond [14]. NAD mediates not only fermentation but also various redox reactions by transferring electrons between its oxidized form, NAD+, and its reduced form, NADH. In particular, NAD plays a very important role as a coenzyme in energy metabolism pathways such as the citric acid cycle and respiratory chain in mitochondria [15].It is said that there are three pathways for synthesizing NAD, the precursor of NAD+, and NAD+ is ultimately produced by amidation with NAD synthetase [16]. NAD is synthesized via the following pathways:

① The kynurenine pathway synthesized from tryptophan [17]

② The salvage pathway by nicotinamide (NAM) [18]

③ The Preiss-Handler pathway by nicotinic acid [19]

By synthesizing NAD in those ways in a multifaceted way, the amount of NAD+ also increases, which may ultimately promote mitochondrial activity. Therefore, it is necessary to increase the blood NAD+ concentration. NAD+ is an intermediate metabolic product from NMN in the salvage pathway [20], and NMN is produced in the body from nicotinamide (NAM) by NAM phosphoribosyl transferase (NAMPT) [21]. Nicotinamide is converted to NMN by NAMPT, and NAD+ is further synthesized from NMN and ATP by MANPT [22]. Therefore, it can be said that NMN is a substance that can increase NAD+, thereby promoting mitochondrial activity and preventing senescence [23,24].It is known that NMN supplementation is an effective treatment for increasing blood NAD+ concentration and preventing senescence. Although it has been shown that exogenously taken NMN is directly absorbed in the mouse ileum via Slc12a8, this has not yet been found in the human digestive tract [25]. Instead, the more widely accepted mechanism in human is that extracellular NMN must be converted to nicotiamide riboside (NR) by dephosphorylation with ectonucleotidase (CD73) prior cellular uptake [26-8], and extracellular NR is taken up into cells via the NR transporters ENT1, ENT2, and ENT4 (Figure 1) [29].In this study, we used a method to entrap NMN into a liposome, which allows it to pass directly through the cell membrane and directly enter the cell. In recent years, many liposomized substances have been seen. A liposome is a spherical vesicle consisting of one or more concentric phospholipid bilayers surrounding an aqueous core [30]. Liposomes are a system that reliably delivers nutrients and medicines into cells because they are not toxic to the body and are degraded in the body [31]. By stabilizing liposomized substances, this technology enhances the effects of nutrients and medicines. It also improves the instability of uptake by cells and tissues, and are attracting attention as a technology that ensures distribution to target sites in the body [32].This technology was applied to the test substance, and NMN was entrapped into a liposome. As shown in Fig. 1, the process of NMN migrating via NR to produce NAD+ for NMN alone is omitted, and by NMN reaching the cells directly, energy use is minimized and no enzymes are used, which increases the concentration of NMN within the cells, and it is thought that more NAD+ is produced.In this study, a double-blind test was conducted on healthy subjects with sufficient informed consent, using three types of NMN: non-liposomal NMN, liposomal NMN and dextrin as a placebo, to measure the amount of NAD+ produced.

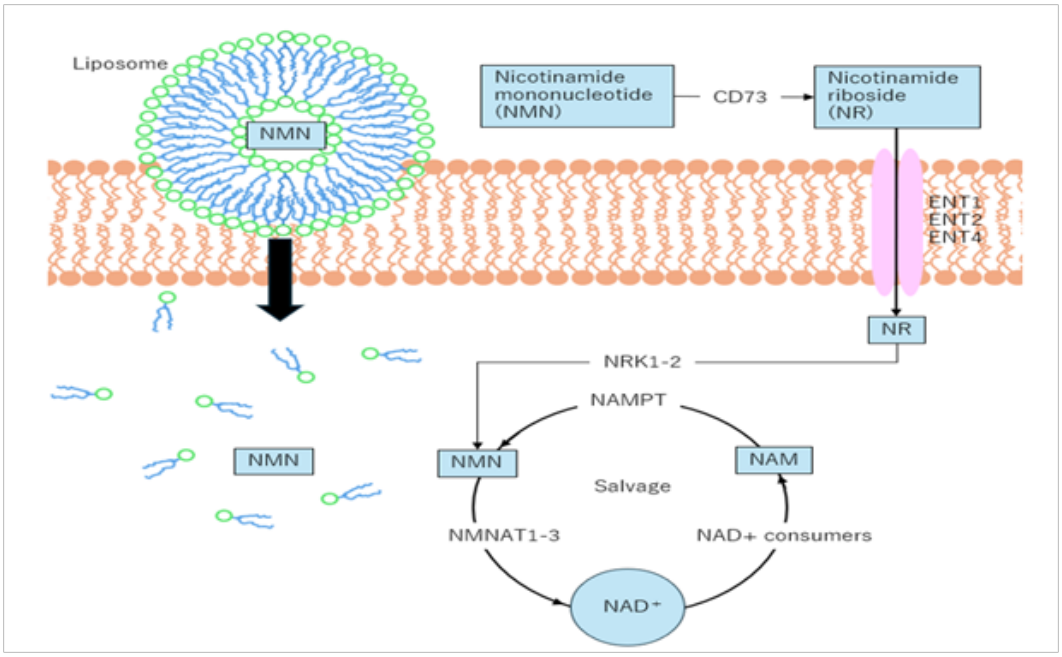

Figure 1: Scheme of NMN and NR uptake into epithelial cells through the cell membrane and the process of NAD+ production.

3. Materials and Methods

3.1. Type of Research

3.1.1 Double-Blind Test

The experimenter selected subjects under certain conditions, randomly packed the test supplements in a plain cardboard box, and provided it directly to the subjects. A document with a description of the product and how to take it was enclosed in the cardboard box, and each subject took the test supplement. After that, blood samples were taken, and MiRTeL Co.LTD (Hiroshima, Japan) was asked to measure NAD+.

3.2. The Conditions Were Set as Follows

[Eligibility criteria]

- Males aged 40 years or older at the time of consent

- Those who fully understand the study plan and are able to give consent

[Exclusion criteria]

- Those with obvious underlying diseases

- Smokers

- Those who drink 20g or more of alcohol per day

- Those with a history of allergies to the study materials

- Those who take NMN, or those who have taken it within the last 14 days

- Those who regularly use health foods that the clinical research director judges may affect the increase in NAD, or those who have taken it within the last 14 days

- Those who cannot maintain their daily lifestyle habits

- Those who are currently participating in other clinical trials, or have participated in other clinical trials within the past 3 months from the date of consent

- Those who plan to participate in other trials during the study period

- Those who are otherwise deemed inappropriate by the clinical research physician

[Discontinuation criteria]

- If an allergy occurs to the study materials

- If consent is withdrawn

-Others who the clinical research director judged to be appropriate for discontinuation due to health hazards or ethical reasons

Number of subjects: 15 cases (5 in each group)

*Rationale for setting: Set as the number of cases possible for statistically analyzable verification research.

3.3. Intervention Method

Fifteen subjects who obtained sufficient informed consent were divided into three groups: 5 in the control group (Group A), 5 in the non-liposomal NMN intake group (Group C), and 5 in the liposomal NMN intake group (Group B). All 15 subjects were instructed to take the test supplement after breakfast for 4 weeks according to the instructions, and blood cell NAD+ tests were performed a total of 4 times: immediately before the start of intake, 1 hour after intake, after the end of the 4-week intake (28 days ± 3 days after intake) and 4 weeks after the end of intake (intake rest period). On the day of the test, no meals were consumed before the test. The test supplement for the placebo group was 100% maltodextrin (4g/day intake). The test supplement for the non-liposomal NMN intake group was a product from another company containing 350 mg of NMN, and the product packaging was covered with a plain label to prevent subjects from identifying what was consumed. The test supplement for the liposomal NMN intake group was also similarly covered with a plain label and used liposomal NMN containing 350 mg of NMN. In addition, liposomal NMN (SINTO liposomal NMN®) was provided free of charge by Premier Anti-Aging Co., Ltd. After the intake was completed and the change in NAD+ was examined. Since this study was a double-blind test, the contents of the test substance in each group were identified after the study was completed. The procedure for the double-blind test is shown in Figure 2.

Table 1: Changes in NAD+ level in the placebo group, liposomal NMN group and non-liposomal NMN group. Values are presented as mean ± standard deviation.

NAD+(μM)	Before intake	1 hour after	4 weeks after	8 weeks after
Placebo	27.9 ±6.8	25.4 ±5.2	26.6 ±3.7	33.5 ±7.5
Liposomal NMN	28.6 ±3.4	32.9 ±7.0	52.5 ±7.9	36.8 ±8.1
Non Liposomal NMN	26.1 ±6.2	24.3 ±3.4	43.5 ±10.6	30.4 ±5.1

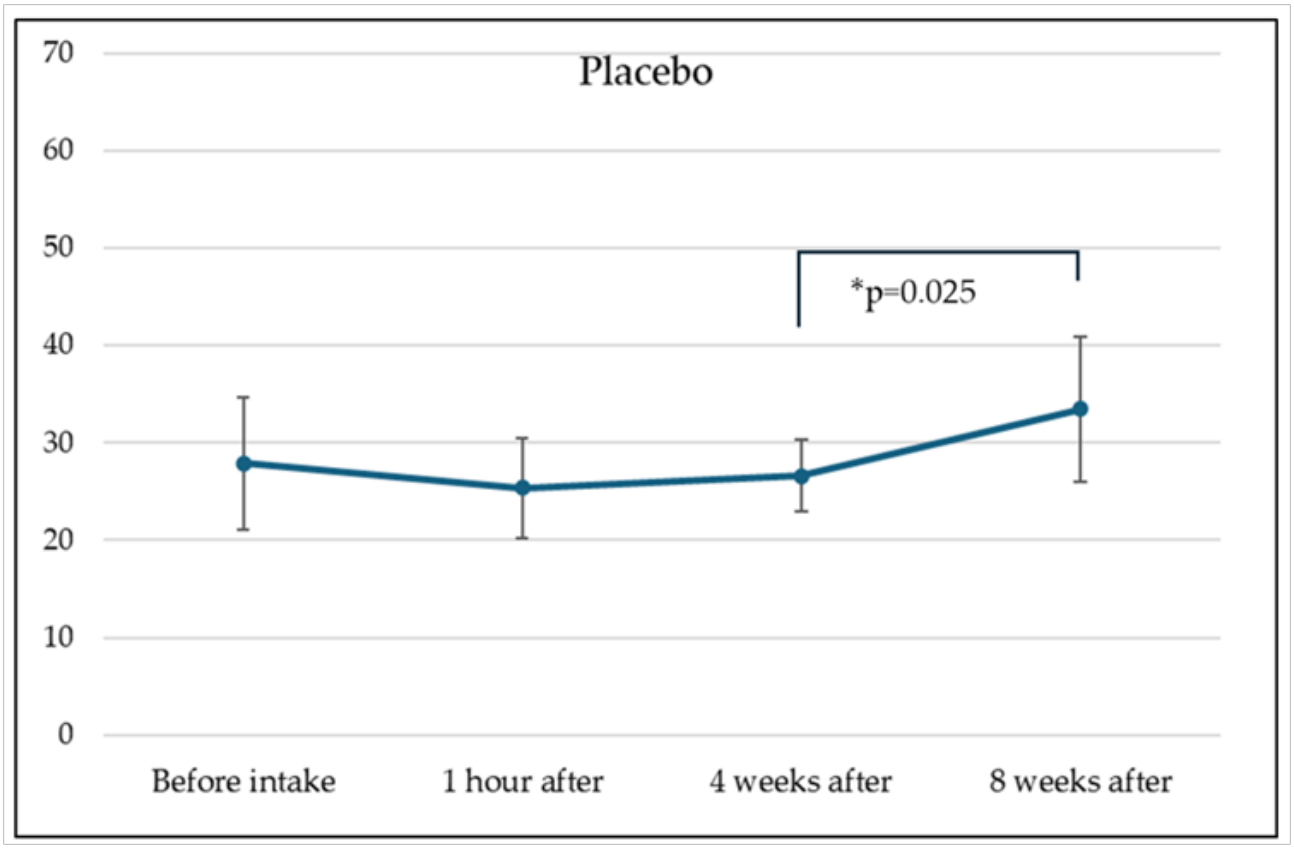

Figure 2: Changes in NAD+ over time for each subject in the placebo group. Values are presented as mean ± standard deviation (μM).

3.4. Statistical Processing

All results of this study were statistically processed using IBM SPSS Statistics (Ver.25) to test for significant differences. The n number was 5 for each group, and the degree of freedom was 2 because the number of subjects in each group was small. In addition, because normal distribution was assumed, Mauchly's test of sphericity was performed, followed by repeated measures analysis of variance, Bonferroni correction, and then paired t-test. In addition, a test for homogeneity of variance was performed for the tests between groups (placebo, non-liposomal NMN, liposomal NMN), followed by one-way analysis of variance and Tukey test to test for significance between groups. The significance level was determined at $P<0.05$.

3.5. Approach to Bias

The reason for including this study was that Japanese men in their 40s and above are at a higher risk of lifestyle-related diseases. On the other hand, Smokers and regular drinkers may have decreased mitochondrial activity from the beginning, and younger people are not significantly different in mitochondrial activity, so they were excluded.

3.6. Ethical Considerations

Ethics approval for this study was obtained through the Ethics Committee of the International Society for clinical medicine longevity. (ISGN_NI10012024).

5. Results

5.1. Changes Over Time in The Placebo Group

Table 1 shows the changes in NAD+ levels over time in the placebo group, liposomal NMN group and non-liposomal NMN group.The following results were obtained when NAD+ was measured for placebo group. No significant differences were observed between the groups before intake, one hour after intake, and four weeks after intake, but NAD+ was significantly elevated four weeks after the end of intake ($p = 0.025$), though the underlying reason remains unclear. The changes over time are shown in a graph in Figure 2.

5.2. Changes over Time in Liposomal NMN

The following results were obtained when liposomal NMN was ingested (Figure 3). No significant difference was observed before and one hour after ingestion, but a tendency for NAD+ to increase was observed. A significant difference for NAD+ to increase was also observed before and four weeks after ingestion ($p = 0.007$). At the same time, a significant difference for NAD+ to increase was observed one hour after and four weeks after ingestion ($p = 0.035$). A significant decrease in NAD+ was observed four weeks after ingestion and four weeks after stopping ingestion (i.e., eight weeks after the start of the study) ($p = 0.043$), but it can be seen that NAD+ increased significantly before and eight weeks after ingestion ($p = 0.043$). This suggests that NAD+ may be produced in large amounts for four weeks even after ingestion is stopped.

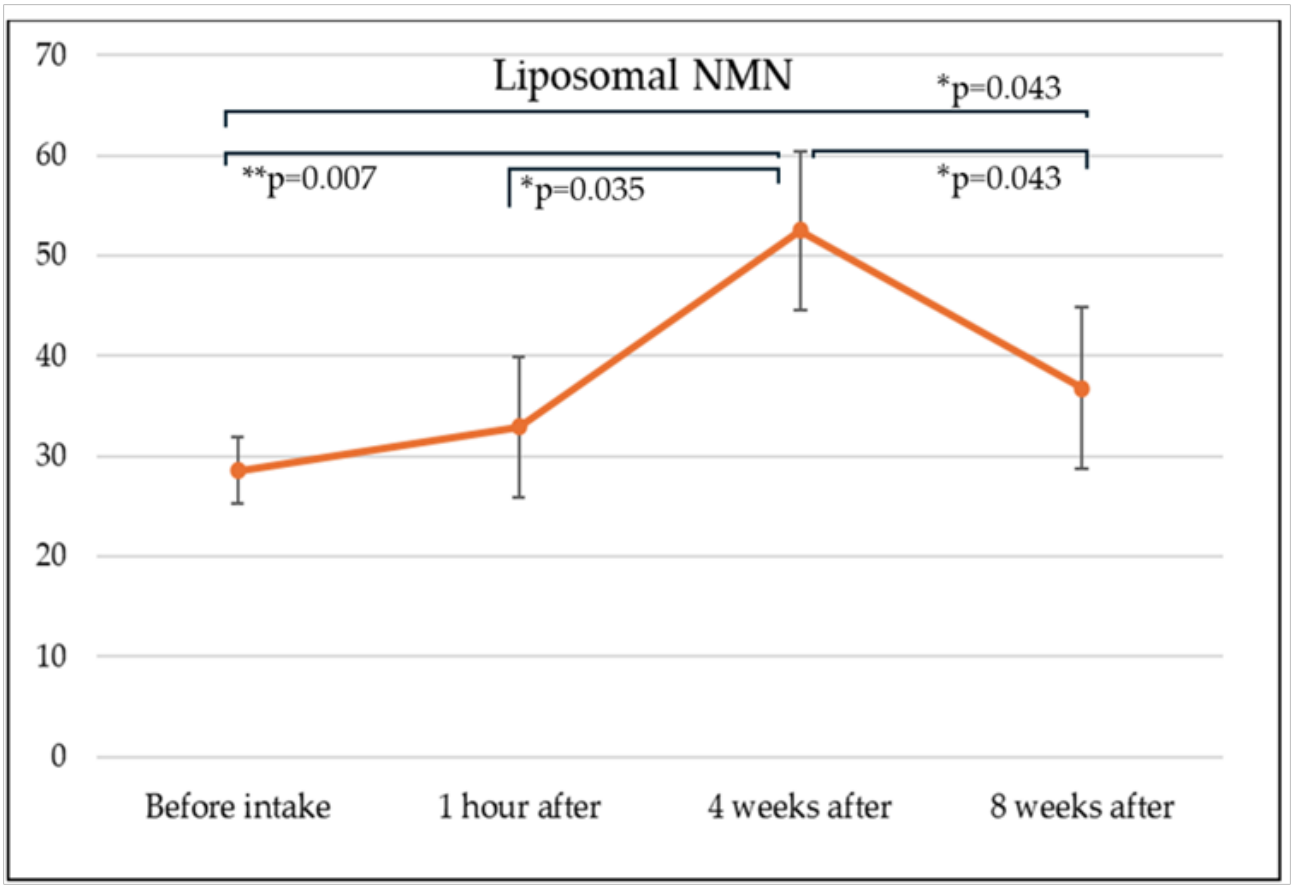

Figure 3: Changes in NAD+ over time for each subject in the liposomal NMN group.

5.3. Changes Over Time with Non-Liposomal NMN

The following results were obtained when non-liposomal NMN was taken (Figure 4). No change was observed in NAD+ before and one hour after ingestion. NAD+ increased significantly one hour after ingestion and four weeks after ingestion ($p=0.024$). A significant decrease in NAD+ was observed four weeks after ingestion and four weeks after cessation of ingestion (i.e., eight weeks after the start of the study) ($p=0.042$). This suggests that the amount of NAD+ produced increases when non-liposomal NMN is taken, but it is possible that NAD+ production may decrease again if ingestion is discontinued, suggesting that non-liposomal NMN may need to be taken continuously.

5.4. Comparison in NAD+ Between Groups after 4 Weeks of Intake

A repeated measures analysis of variance was used to examine the changes in NAD+ after 4 weeks of intake in the placebo, liposomal NMN and non-liposomal NMN groups, followed by a Tukey test. A significant difference was confirmed between the placebo group and the liposomal NMN group ($P<0.05$, $p=0.000$). A significant difference was also confirmed between the liposomal NMN group and the non-liposomal NMN group ($P<0.05$, $p=0.001$). However, a significant difference was not confirmed between the placebo group and the non-liposomal NMN group ($P>0.05$, $p=0.545$). This shows that liposomal NMN promotes the production of NAD+ significantly more than both groups (Figure 5).

5.5. Changes in NAD+ Between Groups 4 Weeks after Cessation of Intake

After stopping the intake of each test substance, participants were asked to live a normal life for 4 weeks, and then the amount of NAD+ in each group was measured. When a Kruskal-Wallis test was performed for each group, $P>0.05$, no significant difference was observed ($p=0.696$). However, between placebo and non-liposomal NMN, the non-liposomal NMN group tended to have a lower level. Similarly, between placebo and liposomal NMN, the liposomal NMN group tended to have a higher level (Figure 6).

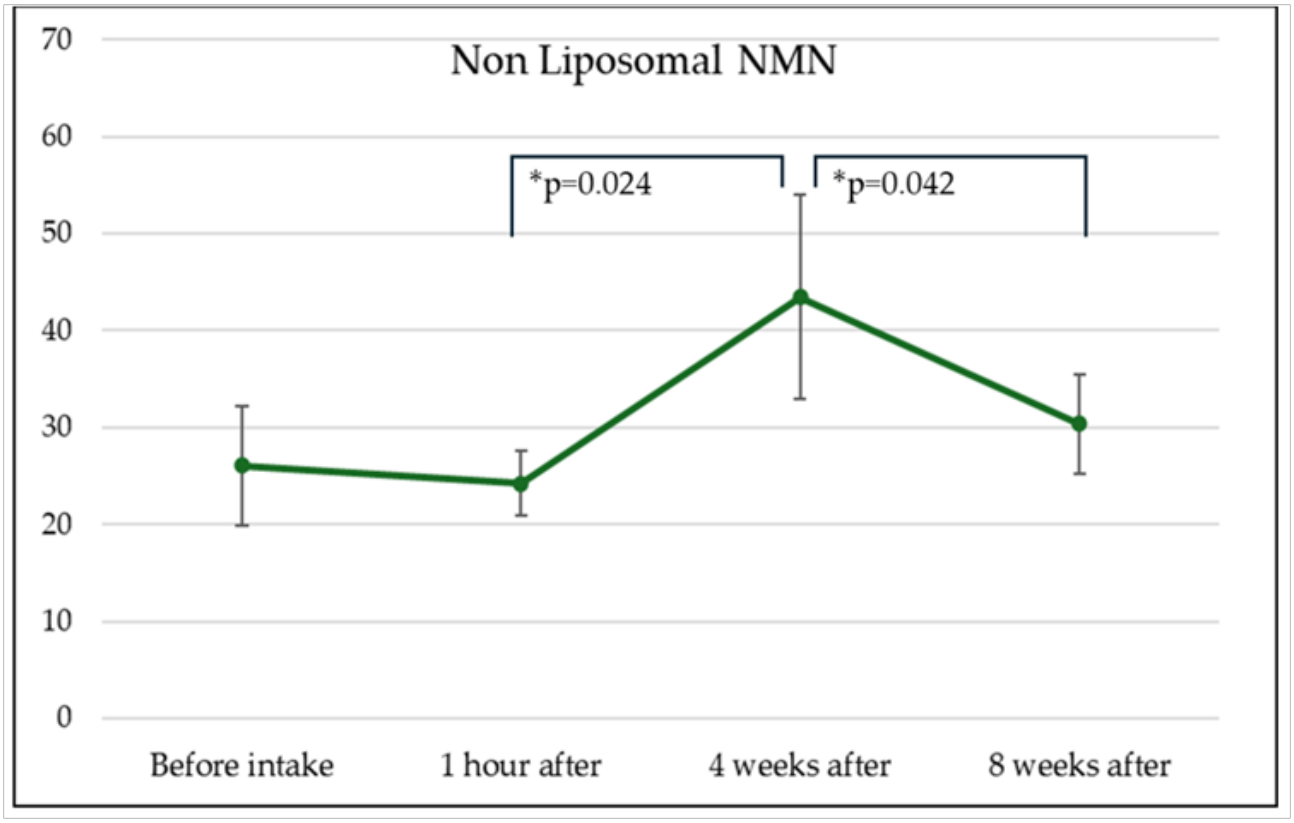

Figure 4: Changes in NAD+ over time for each subject in the non-liposomal NMN group.

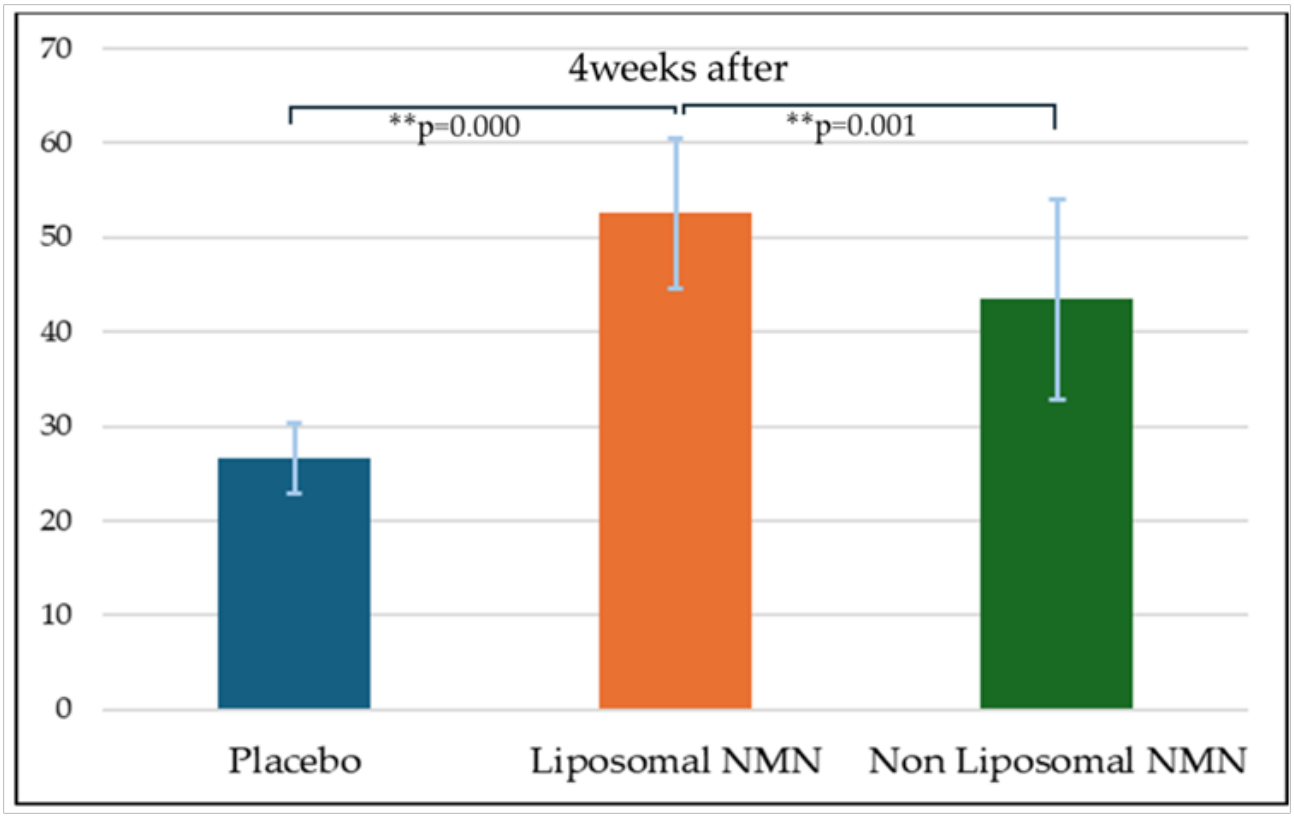

Figure 5: Comparison in NAD+ between groups after 4 weeks of intake.

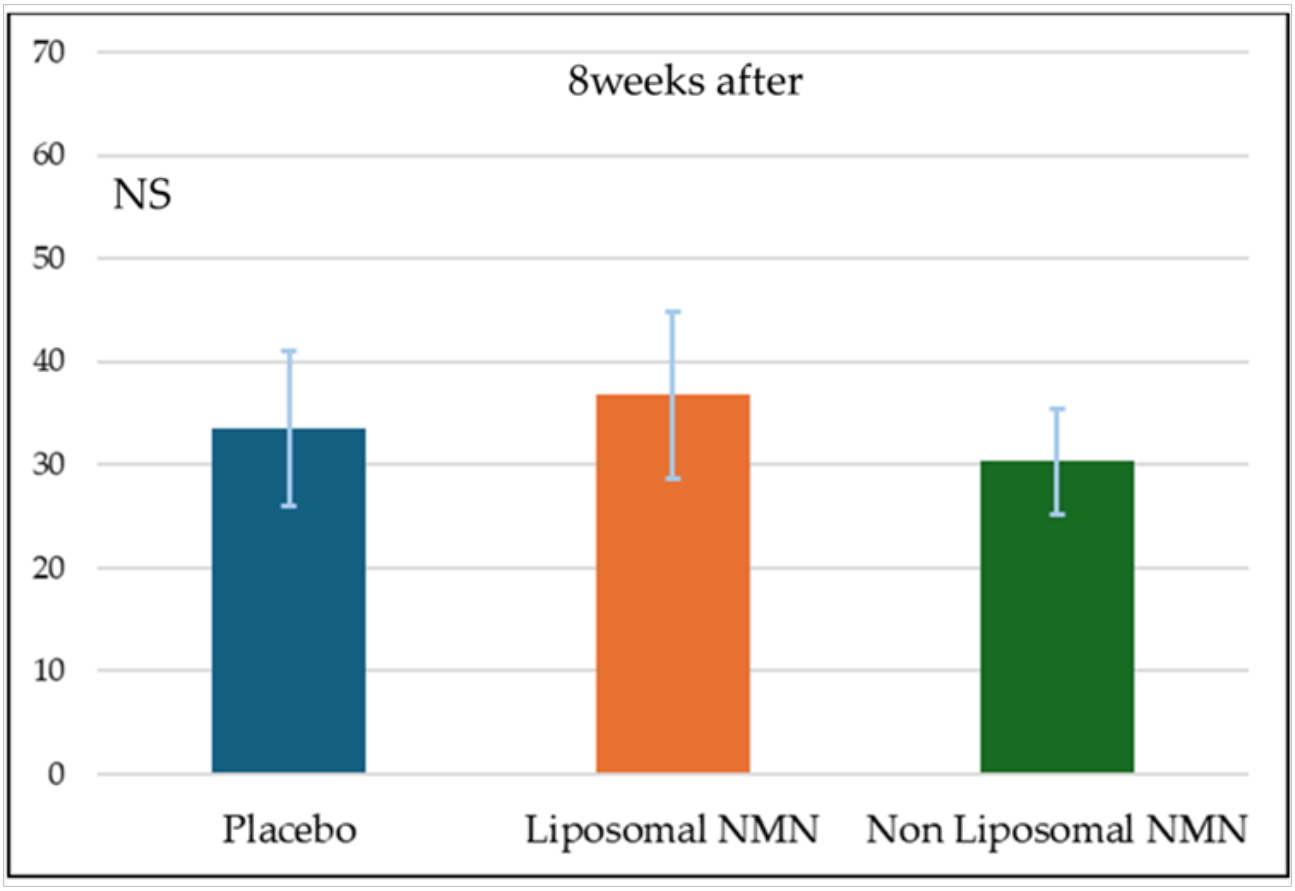

Figure 6: Comparison in NAD+ between groups 4 weeks after cessation of intake.

6. Discussion

In this study, participants were given either a placebo, liposomal NMN, or non-liposomal NMN in a double-blind test, and the amount of NAD+ was measured. It was found that the liposomal NMN group produced significantly more NAD+ in the fourth week of intake. It is said that NAD+ decreases with age [33]. We believe that the results of this study are very meaningful implication as this study showed significant increase of NAD+ significantly increased NAD+ using liposomal NMN. As NAD+ is said to be closely related to mitochondrial activity [34], these results suggest the possibility that liposomal NMN may promote mitochondrial activity. As mitochondrial activity is thought to lead to anti-aging [35], it is believed that there is a good possibility that it could be used to approach the prevention of aging-related diseases coded in ICD-11.In the results of this study, liposomal NMN significantly increased NAD+ compared to non-liposomal NMN. Before non-liposomal NMN is absorbed by cells, it must be converted to NR by dephosphorylation with ectonucleotidase (CD73) [26]. During this process, it is known that extracellular NR is taken up into cells via the NR transporters ENT1, ENT2, and ENT4 (Figure 7)[29]. non-liposomal NMN requires the use of energy due to its one-step process, and at the same time, it is thought that the amount of NMN in the cells may decrease. On the other hand, liposomal NMN is directly absorbed into the cells in its original form through the cell membrane (Figure 8). This allows for efficient NAD+ production within the cells, without the need for energy consumption or a reduction in NMN levels [36]. Therefore, when comparing non-liposomal NMN with liposomal NMN, it is thought that liposomal NMN produces a significantly higher amount of NAD+.Until now, senescence has been regarded as synonymous with aging and has been considered irreversible, but the significance of this research is very great in terms of preventing diseases associated with aging. It is said that there is a causal relationship between aging and mitochondrial activity [37]. NAD was originally discovered as a coenzyme that mediates ethanol fermentation [38]. NAD mediates redox reactions by transferring electrons between the oxidized form NAD+ and the reduced form NADH. In particular, NAD plays an important role as a coenzyme in energy metabolic pathways such as the citric acid cycle and respiratory chain in mitochondria [39]. NAD+ is converted from NMN in our bodies using vitamin B3 as a material [40] , and also NAD+ also plays a role in supplying energy [41]. It has been found that the decrease in NAD with age causes the decline in organ and tissue function and the pathogenesis of aging-related diseases [42]. In the body, mitochondria produces ATP using nutrients and oxygen, and NAD+ is an essential coenzyme for producing ATP, and it is said that a lack of NAD leads to cell death [43].As we age, NAD+ decreases, and oxygen utilization (NAD/NADH ratio) decreases, so the ability to produce the necessary ATP decreases with age [44]. In other words, increasing

NAD+ in the body can be considered to lead be anti-aging [45]. Everyone knows that improving your diet habit is necessary for anti-aging, but it is difficult to suddenly change your diet due to the accumulation of past experiences. It has been pointed out that a sudden change in diet can cause stress (Figure 9), which in turn causes the secretion of stress hormones such as adrenaline and cortisol, which can lead to stress-related diseases [46]. Controlling mental health is very important [47], and acommon problem caused by stress is sleep disorders [48]. A decrease in sleep quality can increase the risk of anxiety and depression, which can lead to a decrease in quality of life. . In this study, there is no direct causal relationship with stress. Science there are reports in various literature that sleep interventions have led to anti-aging [49], the fact that there was no sleep intervention in this study is considered to be of great significant clinically. In a literature search, there were no data that showthat anti-aging can be achieved by taking this test substance and changing lifestyle habits without stress. This study is the first to show that NAD+ can be increased by simply taking the substance, without forcibly changing lifestyle habits, such as intervening in exercise or restricting of diet, and could be useful for anti-aging. It is believed that further elucidation of the mechanism will contribute to the extension of not only average life expectancy but also healthy life expectancy. In addition, the lifestyle-related diseases that are currently a problem are generally phenomena that accompany senescence and are not simply the result of ageing. Preventing the aging phenomenon is considered to be a very important factor because lifestyle-related diseases account for about half of the causes of death. One of the causes of aging is "active oxygen." It is said that reactive oxygen species cause all kinds of lifestyle-related diseases, and therefore it is necessary to remove them. However, since reactive oxygen species also play a role in biological defense, there are both positive and negative aspects to their removal [50,51]. For example, neutrophils release reactive oxygen species to process antigens, and without the presence of reactive oxygen species, it would be difficult to remove the antigens [52]. However, it is known that excessive production of reactive oxygen species attacks normal cells and has a negative effect on the body [53]. There are four types of reactive oxygen species: superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen, and hydroxyl radical is said to be the most malignant [54]. In this situation, NMN, which also has an antioxidant effect [55], is thought to be able to remove a moderate amount of reactive oxygen species and maintain homeostasis in the body.

It is known that reactive oxygen species not only attack normal cells, but also act on mitochondria [56]. Therefore, it can be said that damaging mitochondria inevitably reduces the production of NAD+. Currently, mitochondrial activity is said to be deeply related to anti-aging [57].

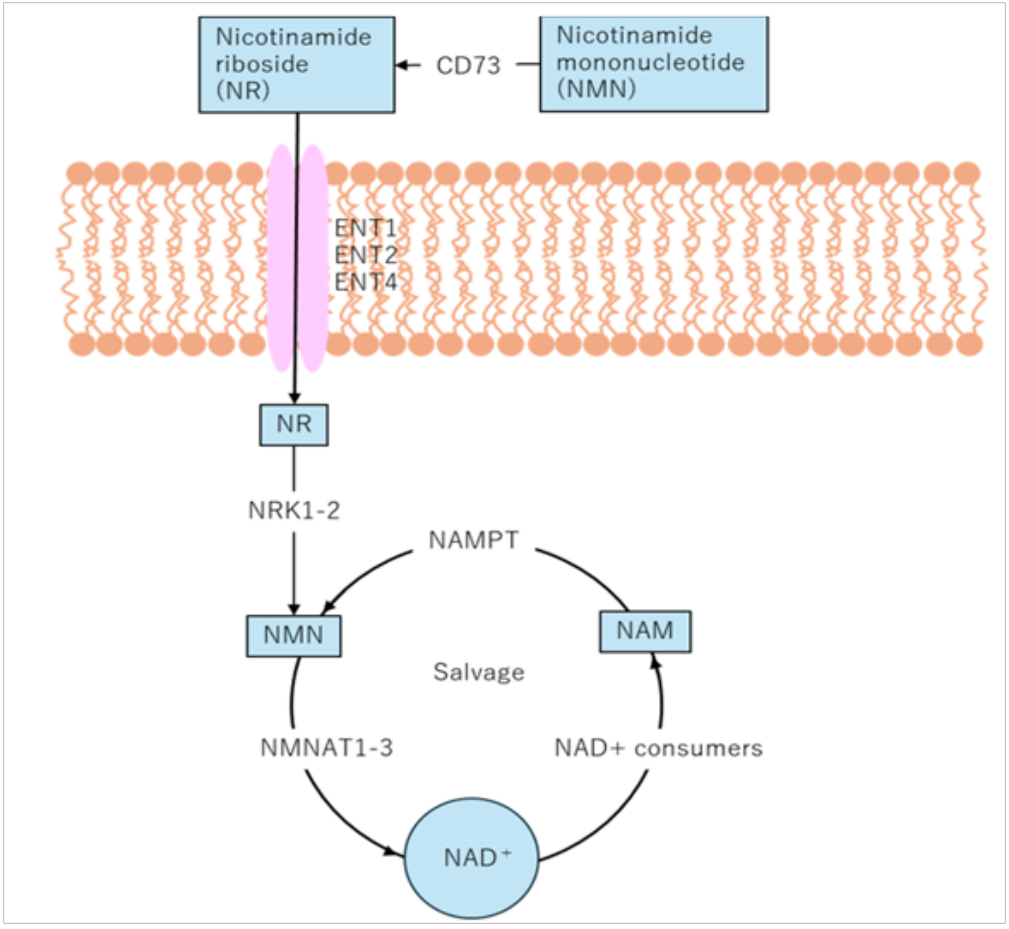

Figure 7: The process of NAD+ production during intracellular delivery of non-liposomal NMN.

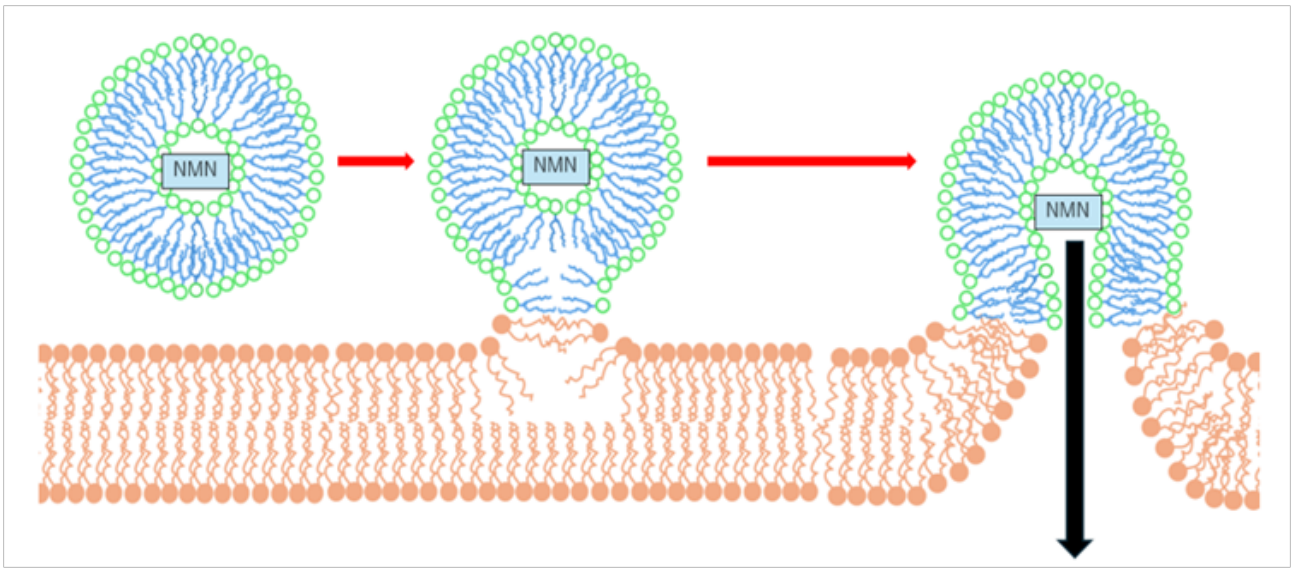

Figure 8: NMN entry scheme by membrane fusion of liposomes into small intestinal mucosal cells.

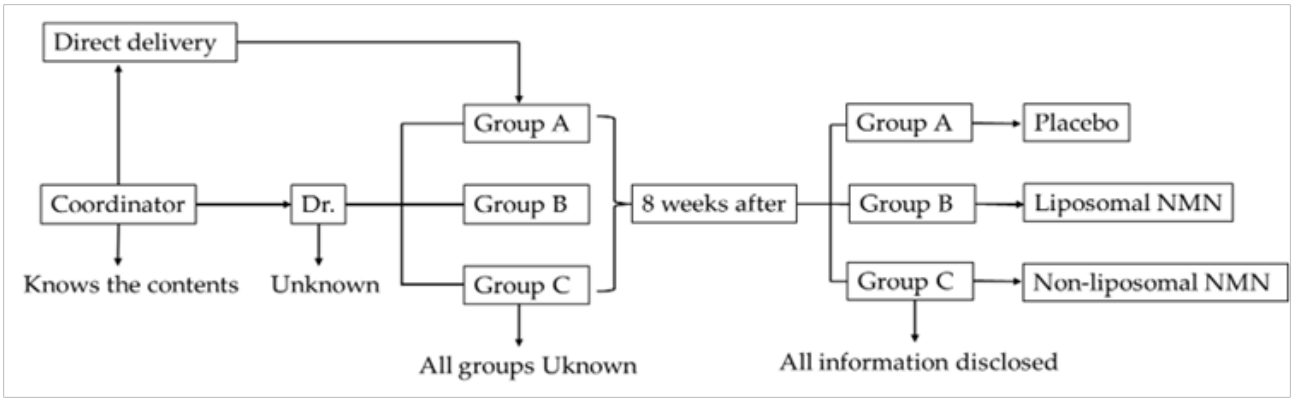

Figure 9: Double-blind test procedure.

6.1. Mitochondrial Activation has The Following Effects:

① Energy production [58]

② Increased immune function [59]

③ Antioxidant effect [60]

④ Treatment of age-related diseases [61]

⑤ Suppression of aging-related inflammation [62]

These findings show that activating mitochondria is a very important factor in anti-aging. There are various substances that activate mitochondria, but this time we focused on NMN because it is said to have mitochondrial activity [63]. In addition, NMN is said to be deeply involved in maintaining and improving health by acting on the sirtuin gene, a longevity gene [64]. Furthermore, previous research has shown that NMN enhances AMP-activated protein kinase (AMPK) [65]. AMPK is an energy sensor in the body and a serine/threonine kinase that works to maintain homeostasis of sugar and lipid metabolism [66]. It is said that activation of AMPK regulates energy metabolism and maintains energy homeostasis, and it has been attracting attention as a potential therapeutic agent for metabolic diseases including type 2 diabetes and cancer [67]. Energy is essential for human life, and the source of energy is ATP, which is generated when ATP is hydrolyzed and converted to adenosine diphosphate (ADP) [68]. AMPK regulates this ATP level to maintain homeostasis and is expected to be effective against metabolic diseases such as cancer, type 2 diabetes and obesity [69-71]. In other words, it is expected that increasing AMPK activity can prevent lifestyle-related diseases including cancer and so on.Taking NMN externally is considered to be very meaningful. Among the different forms, liposomal NMN, which was able to produce more NAD+ than non-liposomal NMN, can be considered particularly useful. Not only does it help in preventing lifestyle-related diseases, but it also for anti-aging. As human beings evolve, our lifestyles change, but it is thought that this test substance could improve the internal environment of the body, leading to anti-aging. By maintaining a stable internal environment and proper homeostasis, it could be possible for a person to regain their original, healthy, and optimal state. From this perspective, the significance of this research is considered to be extremely great.

7. Conclusions

In this study, participants were either a placebo, liposomal NMN or non-liposomal NMN in a double-blind test, and the amount of NAD+ was measured. It was found that the liposomal NMN group produced significantly more NAD+ at the fourth week of intake. However, when NMN intake was stopped, the amount of NAD+ production significantly decreased at the fourth week. Other studies have also reported that when healthy adults were given NMN for a certain period of time and then stopped, the amount of NAD+ production, which had been elevated, decreased [72]. From these findings, it is clear that continuous intake of NMN is important to increase NAD+ production in the body. However, it is not clear to what extent increasing NAD+ is beneficial for mitochondrial activity, removal of active oxygen, AMPK activity, etc., and whether it is beneficial for anti-aging effects and prevention of aging-related diseases. In addition, this study was a limited exploratory intervention study of 8 weeks for 15 subjects, and future research is needed on the effects of taking it for a longer period of time at large scale.

References

1. Wang C, Ni W, Yao Y, Just A, Heiss J, Wei Y, Gao X. DNA methylation-based biomarkers of age acceleration and all-cause death, myocardial infarction, stroke, and cancer in two cohorts: The NAS, and KORA F4. EbioMedicine. 2021;63:103151.
2. Calimport SRG, Bentley BL. Aging Classified as a Cause of Disease in ICD-11. Rejuvenation Res. 2019;22(4):281.
3. Zhu D, Li X, Tian Y. Mitochondrial-to-nuclear communication in aging: an epigenetic perspective. Trends Biochem Sci. 2022;47(8):645-659.
4. Sun N, Youle RJ, Finkel T. The Mitochondrial Basis of Aging. Mol Cell. 2016;61(5):654-666.
5. Burtscher J, Soltany A, Visavadiya NP, Burtscher M, Millet GP, Khoramipour K, Khamoui AV. Mitochondrial stress and mitokines in aging. Aging Cell. 2023;22(2):e13770.
6. Cantó C, Menzies KJ, Auwerx J. NAD(+) Metabolism and the Control of Energy Homeostasis: A Balancing Act between Mitochondria and the Nucleus. Cell Metab. 2015;22(1):31-53.
7. Massudi H, Grant R, Braidy N, Guest J, Farnsworth B, Guillemin GJ. Age-associated changes in oxidative stress and NAD+ metabolism in human tissue. PLoS One. 2012;7(7): e42357.
8. Shen S, Shen M, Kuang L, Yang K, Wu S, Liu X. SIRT1/SREBPs-mediated regulation of lipid metabolism. Pharmacol Res. 2024;199:107037.
9. Verdin E. NAD^+ in aging, metabolism, and neurodegeneration. Science. 2015;350(6265):1208-13.
10. Imai S, Guarente L. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 2014;24(8):464-71.
11. Chu X, Raju RP. Regulation of NAD+ metabolism in aging and disease. Metabolism. 2022;126:154923.
12. Xue X, Miao Y, Wei Z. Nicotinamide adenine dinucleotide metabolism: driving or counterbalancing inflammatory bowel disease? FEBS Lett. 2023;597(9):1179-1192.
13. Anderson KA, Madsen AS, Olsen CA, Hirschey MD. Metabolic control by sirtuins and other enzymes that sense NAD, NADH, or their ratio. Biochim Biophys Acta Bioenerg. 2017;1858(12):991-998.
14. Shen Q, Zhang SJ, Xue YZ, Peng F, Cheng DY, Xue YP. Biological synthesis of nicotinamide mononucleotide. Biotechnol Lett. 2021;43(12):2199-2208.
15. Covarrubias AJ, Perrone R, Grozio A, Verdin E. NAD+ metabolism and its roles in cellular processes during ageing. Nat Rev Mol Cell Biol. 2021;22(2):119-141.
16. Zalkin H. NAD synthetase. Methods Enzymol. 1985;113:297-302.
17. Castro-Portuguez R, Sutphin GL. Kynurenine pathway, NAD+ synthesis, and mitochondrial function: Targeting tryptophan metabolism to promote longevity and healthspan. Exp Gerontol. 2020;132:110841.
18. Kennedy BE, Sharif T, Martell E, Dai C, Kim Y, Lee PW, Gujar SA. NAD+ salvage pathway in cancer metabolism and therapy. Pharmacol Res. 2016;114:274-283.
19. Audrito V, Messana VG, Deaglio S. NAMPT and NAPRT: Two Metabolic Enzymes with Key Roles in Inflammation. Front Oncol. 2020;10:358.
20. Niu KM, Bao T, Gao L, Ru M, Li Y, Jiang L, Ye C. The Impacts of Short-Term NMN Supplementation on Serum Metabolism, Fecal Microbiota, and Telomere Length in Pre-Aging Phase. Front Nutr. 2021;8:756243.
21. Jayaram HN, Kusumanchi P, Yalowitz JA. NMNAT expression and its relation to NAD metabolism. Curr Med Chem. 2011;18(13):1962-72.
22. Grolla AA, Miggiano R, Di Marino D, Bianchi M, Gori A, Orsomando G, Gaudino F. A nicotinamide phosphoribosyltransferase-GAPDH interaction sustains the stress-induced NMN/NAD+ salvage pathway in the nucleus. J Biol Chem. 2020;295(11):3635-3651.
23. Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, Sasaki Y. Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice. Cell Metab. 2016;24(6):795-806.
24. Kawamura T, Mori N, Shibata K. β-Nicotinamide Mononucleotide, an Anti-Aging Candidate Compound, Is Retained in the Body for Longer than Nicotinamide in Rats. J Nutr Sci Vitaminol (Tokyo). 2016;62(4):272-276.
25. Schmidt MS, Brenner C. Absence of evidence that Slc12a8 encodes a nicotinamide mononucleotide transporter. Nat Metab. 2019;1(7):660-661.
26. Mateuszuk Ł, Campagna R, Kutryb-Zając B, Kuś K, Słominska EM. Reversal of endothelial dysfunction by nicotinamide mononucleotide via extracellular conversion to nicotinamide riboside. Biochem Pharmacol. 2020;178:114019.

27. Fletcher RS, Ratajczak J, Doig CL, Oakey LA, Callingham R, Da Silva Xavier G. Nicotinamide riboside kinases display redundancy in mediating nicotinamide mononucleotide and nicotinamide riboside metabolism in skeletal muscle cells. Mol Metab. 2017;6(8):819-832.
28. Fragola G, Mabb AM, Taylor-Blake B, Niehaus JK, Chronister WD, Mao H. Deletion of Topoisomerase 1 in excitatory neurons causes genomic instability and early onset neurodegeneration. Nat Commun. 2020; 11(1):1962.
29. Kropotov A, Kulikova V, Nerinovski K, Yakimov A, Svetlova M, Solovjeva L. Equilibrative Nucleoside Transporters Mediate the Import of Nicotinamide Riboside and Nicotinic Acid Riboside into Human Cells. Int J Mol Sci. 2021;22(3):1391.
30. Guimarães D, Cavaco-Paulo A, Nogueira E. Design of liposomes as drug delivery system for therapeutic applications. Int J Pharm. 2021;601:120571.
31. Fan Y, Marioli M, Zhang K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. J Pharm Biomed Anal. 2021;192:113642.
32. Man F, Gawne PJ, T M de Rosales R. Nuclear imaging of liposomal drug delivery systems: A critical review of radiolabelling methods and applications in nanomedicine. Adv Drug Deliv Rev. 2019;143:134-160.
33. Clement J, Wong M, Poljak A, Sachdev P, Braidy N. The Plasma NAD+ Metabolome Is Dysregulated in "Normal" Aging. Rejuvenation Res. 2019;22(2):121-130.
34. Imai S, Guarente L. NAD+ and sirtuins in aging and disease. Trends Cell Biol. 2014;24(8):464-71.
35. Li Y, Tian X, Luo J, Bao T, Wang S, Wu X. Molecular mechanisms of aging and anti-aging strategies. Cell Commun Signal. 2024;22(1):285.
36. Patel HM, Tuzel NS, Stevenson RW. Intracellular digestion of saturated and unsaturated phospholipid liposomes by mucosal cells. Possible mechanism of transport of liposomally entrapped macromolecules across the isolated vascularly perfused rabbit ileum. Biochim Biophys Acta. 1985;839(1):40-9.
37. Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV. Mitochondrial aging and age-related dysfunction of mitochondria. Biomed Res Int. 2014;238463.
38. Harden A, Young WJ. The alcoholic herment of yeast-juice. Proc. R Soc Lond B. 1906; 77: 405-420.
39. Sauve AA. NAD+ and vitamin B3: from metabolism to therapies. J Pharmacol Exp Ther. 2008;324(3):883-93.
40. Yoshino J, Mills KF, Yoon MJ, Imai S. Nicotinamide mononucleotide, a key NAD(+) intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab. 2011;14(4):528-36.
41. Roh E, Myoung Kang G, Young Gil S, Hee Lee C, Kim S, Hong D. Effects of Chronic NAD Supplementation on Energy Metabolism and Diurnal Rhythm in Obese Mice. Obesity (Silver Spring). 2018;26(9):1448-1456.
42. Bhasin S, Seals D, Migaud M, Musi N, Baur JA. Nicotinamide Adenine Dinucleotide in Aging Biology: Potential Applications and Many Unknowns. Endocr Rev. 2023;44(6):1047-1073.
43. Zhang T, Liu Q, Gao W, Sehgal SA, Wu H. The multifaceted regulation of mitophagy by endogenous metabolites. Autophagy. 2022;18(6):1216-1239.
44. Gao X, Yu X, Zhang C, Wang Y, Sun Y, Sun H. Telomeres and Mitochondrial Metabolism: Implications for Cellular Senescence and Age-related Diseases. Stem Cell Rev Rep. 2022;18(7):2315-2327.
45. Johnson S, Imai SI. NAD + biosynthesis, aging, and disease. F1000Res. 2018;7:132.
46. Noushad S, Ahmed S, Ansari B, Mustafa UH, Saleem Y, Hazrat H. Physiological biomarkers of chronic stress: A systematic review. Int J Health Sci (Qassim). 2021;15(5):46-59.
47. Scott AJ, Webb TL, Martyn-St James M, Rowse G, Weich S. Improving sleep quality leads to better mental health: A meta-analysis of randomised controlled trials. Sleep Med Rev. 2021; 60:101556.
48. Nelson KL, Davis JE, Corbett CF. Sleep quality: An evolutionary concept analysis. Nurs Forum. 2022;57(1):144-151.
49. Huang D, Wang S. Association Between the Anti-Aging Protein Klotho and Sleep Duration in General Population. Int J Gen Med. 2021;14:10023-10030.
50. Herb M, Schramm M. Functions of ROS in Macrophages and Antimicrobial Immunity. Antioxidants (Basel). 2021;10(2):313.
51. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiol Rev. 2014;94(3):909-50.
52. Rodrigues LC, Cerri DG, Marzocchi-Machado CM, Cummings RD, Stowell SR, Dias-Baruffi M. Detection of Reactive Oxygen Species in Human Neutrophils Under Various Conditions of Exposure to Galectin. Methods Mol Biol. 2022;2442:549-564.
53. Shi T, Dansen TB. Reactive Oxygen Species Induced p53 Activation: DNA Damage, Redox Signaling, or Both? Antioxid Redox Signal. 2020;33(12):839-859.
54. Jakubczyk K, Dec K, Kałduńska J, Kawczuga D, Kochman J, Janda K. Reactive oxygen species - sources, functions, oxidative damage. Pol Merkur Lekarski. 2020;48(284):124-127.
55. Zhang R, Shen Y, Zhou L, Sangwung P, Fujioka H, Zhang L, Liao X. Short-term administration of Nicotinamide Mononucleotide preserves cardiac mitochondrial homeostasis and prevents heart failure. J Mol Cell Cardiol. 2017;112:64-73.
56. Murphy MP. Understanding and preventing mitochondrial oxidative damage. Biochem Soc Trans. 2016;44(5):1219-1226.
57. Zhao R, Dong C, Liang Q, Gao J, Sun C, Gu Z, Zhu Y. Engineered Mitochondrial Transplantation as An Anti-Aging Therapy. Aging Dis. 2024.
58. Li PA, Hou X, Hao S. Mitochondrial biogenesis in neurodegeneration. J Neurosci Res. 2017;95(10):2025-2029.

59. Saha T, Dash C, Jayabalan R, Khiste S, Kulkarni A, Kurmi K. Intercellular nanotubes mediate mitochondrial trafficking between cancer and immune cells. Nat Nanotechnol. 2022 Jan;17(1):98-106.
60. Wang P, Li CG, Qi Z, Cui D, Ding S. Acute exercise stress promotes Ref1/Nrf2 signalling and increases mitochondrial antioxidant activity in skeletal muscle. Exp Physiol. 2016;101(3):410-20.
61. Nguyen TT, Wei S, Nguyen TH, Jo Y, Zhang Y, Park W. Mitochondria-associated programmed cell death as a therapeutic target for age-related disease. Exp Mol Med. 2023;55(8):1595-1619.
62. Bharath LP, Agrawal M, McCambridge G, Nicholas DA, Hasturk H, Liu J. Metformin Enhances Autophagy and Normalizes Mitochondrial Function to Alleviate Aging-Associated Inflammation. Cell Metab. 2020;32(1):44-55.e6.
63. Wang H, Sun Y, Pi C, Yu X, Gao X, Zhang C. Nicotinamide Mononucleotide Supplementation Improves Mitochondrial Dysfunction and Rescues Cellular Senescence by NAD+/Sirt3 Pathway in Mesenchymal Stem Cells. Int J Mol Sci. 2022;23(23):14739.
64. Nakajo T, Kitajima N, Katayoshi T, Tsuji-Naito K. Nicotinamide mononucleotide inhibits oxidative stress-induced damage in a SIRT1/NQO-1-dependent manner. Toxicol In Vitro. 2023;93:105683.
65. Tabibzadeh S. From genoprotection to rejuvenation. Front Biosci (Landmark Ed). 2021;26(1):97-162.
66. Yuichi YOKOYAMA, Kazuhiro IGUCHI, Shigeyuki USUI, Kazuyuki HIRANO. Regulation of Glucose and Lipid Metabolism via AMPK. The annual proceedings of Gifu Pharmaceutical University 201; 68-74.
67. Carling D. AMPK signalling in health and disease. Curr Opin Cell Biol. 2017;45:31-37.
68. Boyer PD, Chance B, Ernster L, Mitchell P, Racker E, Slater EC. Oxidative phosphorylation and photophosphorylation. Annu Rev Biochem. 1977;46:955-66.
69. Cool B, Zinker B, Chiou W, Kifle L, Cao N, Perham M. Identification and characterization of a small molecule AMPK activator that treats key components of type 2 diabetes and the metabolic syndrome. Cell Metab. 2006;3(6):403-16.
70. Giordanetto F, Karis D. Direct AMP-activated protein kinase activators: a review of evidence from the patent literature. Expert Opin Ther Pat. 2012;22(12):1467-77.
71. Xiao B, Sanders MJ, Carmena D, Bright NJ, Haire LF, Underwood E, Patel BR. Structural basis of AMPK regulation by small molecule activators. Nat Commun. 2013;4:3017.
72. Okabe K, Yaku K, Uchida Y, Fukamizu Y, Sato T, Sakurai T. Oral administration of nicotinamide mononucleotide is safe and efficiently increases blood nicotinamide adenine dinucleotide levels in healthy subjects. Front Nutr. 2022;9:868640.